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Ballintijn, C.M.; Bamford, O.S.

Published in:
Journal of Experimental Biology

Publication date:
1975

J. exp. Biol. (1975), 62, 99–114
With 9 figures
Printed in Great Britain

PROPRIOCEPTIVE MOTOR CONTROL IN FISH RESPIRATION

BY C. M. BALLINTIJN AND O. S. BAMFORD*

Zoological Laboratory of the University of Groningen, Kerklaan 30, Haren, The Netherlands

(*Received 5 July 1974*)

SUMMARY

The response of single respiratory neurones in the medulla oblongata of carp to short twitches of individual respiratory muscles were analysed. The muscle contractions were obtained through automatic electrical stimulation and could be consistently elicited in a predetermined phase relation to the ventilatory cycle. The results show that, apart from nerve cells which take part in long-term processing of proprioceptive information from several sources, neurones also exist which possess the properties of elements of a peripheral proprioceptive control loop such as tension receptor neurones, length or stretch receptor neurones and motor neurones.

INTRODUCTION

In the past, experiments have been carried out on both teleosts and elasmobranchs to elucidate the function of mechanoreceptors in the respiratory system. Satchell & Way (1962) studied receptors on the branchial processes of the dogfish and the role they play in the regulation of respiration, and Sutterlin & Saunders (1969) studied the mechanoreceptors on the gills of the sea raven.

In general, the interest of investigators has been focused on long-term processes such as the contribution of mechanoreception and proprioception to the development of the respiratory rhythm, and the influence of proprioceptive signals of one breath upon the execution of the following (Ballintijn, 1969*b*; von Baumgarten & Salmoiraghi, 1962; Satchell, 1959, 1961; Serbenyuk, 1964, 1965; Serbenyuk, Shishov & Kiprian, 1959; Shelton, 1970).

A further aspect of proprioceptive control in teleosts is whether there is a direct proprioceptive reflex control of the activity of the respiratory pump muscles.

In mammals it has been shown that the respiratory centre, contrary to earlier theory, does not directly activate the α motor neurones of the intercostal muscles, which only receive subliminal respiratory drive potentials, modifying their sensitivity. The γ motor neurones of the muscle spindles, however, respond to the central drive potentials and via the γ reflex loop activate the α motor neurones. Thus control of the respiratory movements is exerted via the proprioceptive γ reflex loop in which external disturbances of the movements as a result of postural and respiratory demands are

* Present address: Zoology Department, University of British Columbia, Canada.

compensated. (For an extensive account and survey of the literature see Granit, 1970, and Newsom Davis, 1970.)

Histological observations on some teleost muscles (Bone, 1964) show that such complicated organs as the muscle spindles of higher vertebrates are not present. Muscles making complicated movements, however, are equipped with free nerve endings with a sensory function, both in the muscle and in the tendon. Thus the elements for a simple proprioceptive control loop seem to be present in fish. To our knowledge no histological data are available for the respiratory muscles.

Electrophysiological research on proprioceptive motor control in fish respiration is complicated by the fact that, unlike the situation in mammals, the motor neurones of the respiratory pump muscles are located in the medulla oblongata near the central respiratory neurones. Nevertheless it has been possible to demonstrate that proprioceptive regulation of the respiratory movements occurs in fish on the following evidence.

Experimental disturbances of the respiratory movements are immediately followed by compensatory changes in the electromyogram of the respiratory muscles influenced by the disturbance (Ballintijn, 1969*a*). The muscle antagonized by the disturbance show an increase in activity, those assisted show a diminution. The fact that the compensation is without delay and is adapted to the situation for each muscle individually indicates that it is caused by proprioceptive control rather than by oxygen lack through impaired ventilation. In the latter case the reaction would develop only after a delay, and an increased respiratory effort due to suffocation would result in an overall augmentation of respiratory muscle activity.

Recording experiments also showed that neurones in the medulla oblongata process proprioceptive information and often stop firing when they are deprived of proprioceptive input (Ballintijn, 1969*b*, 1972).

The aim of the present paper is to identify further the cells that process proprioceptive information and to demonstrate that a number of them have properties identical with those expected from the motor neurones, tension receptor neurones and length receptor neurones of a peripheral proprioceptive reflex loop.

MATERIAL AND METHODS

The experiments were performed in the following way. The electrical activity of single respiratory neurones in the medulla oblongata was recorded. After determination of the spontaneous activity of the cell under observation, respiratory pump muscles were stimulated one at a time with short bursts of electrical pulses during predetermined parts of the respiratory cycle. The time relation between the stimulus bursts and the respiratory cycle could be varied. The effect of the resulting short muscle twitches upon the activity of the respiratory neurone was analysed. In each situation a record was made with: 1 min. stimulus off, 1 min. stimulus on, 1 min. stimulus off.

For all experiments carp (*Cyprinus carpio* L.) were used and these were kept in the basement of the building in concrete tanks with running tap water and in large stock ponds outside. The fish measured 19–23 cm.

During the experiments the fish were anaesthetized through addition of MS 222

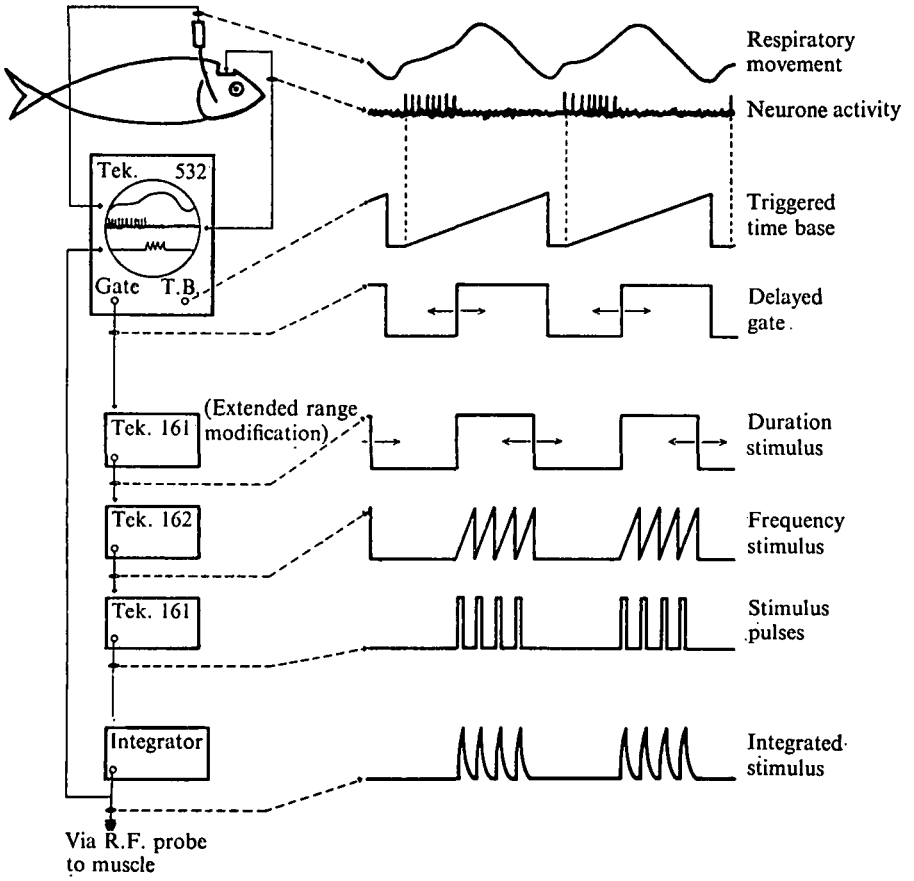

Fig. 1. Schematic diagram of the apparatus used for generating the muscle stimulus and timing it with respect to the recorded neurone activity. For further explanation see text.

to the water in the experimental tank (30–50 mg/l) and were fixed in a clamp. A hole was made in the top of the skull exposing the cerebellum, facial and vagal lobes. The level of the water in the tank was kept just below the border of the skull, but high enough to cover the opercular slits completely.

Electrical activity of individual neurones in the medulla oblongata was recorded with bipolar glass-coated silver electrodes.

The respiratory muscles were stimulated through monopolar stainless steel electrodes of 150–250 μm placed far apart in each muscle. The stimulus, a short burst of impulses, was obtained from a Tektronix 160 series generator. To reduce the stimulus artifacts picked up by the brain recording electrodes, the impulses were integrated with a simple R.C. filter and coupled to the muscle through a radio frequency isolation unit. In combination with the bipolar recording technique, stimulus artifacts could thus usually be kept very small.

The phase relation between the stimulus and the respiratory activity of the fish could be controlled as follows (Fig. 1). Every first spike of the burst of activity recorded from a respiratory cell triggered the time base of a Tektronix 532 oscilloscope.

The sweep was adjusted to a slightly shorter duration than one respiratory cycle. The onset of the 'delayed gate' of this oscilloscope served as trigger for the stimulus generator. Thus the phase relation between stimulus and respiratory activity was automatically kept constant while the onset of the stimulus could be adjusted throughout the respiratory cycle using the 'gate delay' control of the oscilloscope. In this way it was possible to stimulate in phase or in antiphase with the normal activity of any respiratory neurone or muscle.

Apart from synchronizing the signals, the oscilloscope also served as a monitor in displaying the neurone activity and the stimulus signal.

As a measure of respiration in general, the movements of the hyomandibula or the extreme anterior border of the operculum which closely follows the hyomandibula movements, were recorded with a very light mechano-electrical transducer. This particular recording site was chosen because it gives the best overall impression of respiration, as the hyomandibula plays a role in the movements of the buccal and of the opercular pumps.

All the relevant data (nerve cell activity, the stimulus pulses and the movement signal) were recorded on a CEC instrumentation tape recorder.

The tapes were analysed on a PDP 9 computer of the University Computing Centre.

RESULTS

Of the 53 cells studied in 33 fish, about one-third changed their firing characteristics as a reaction to electrical stimulation of the respiratory muscles. These units are examined in this section, divided into groups according to the phase of respiration in which the neurone under observation was active. This is convenient because, as will be shown in the discussion, the timing of the activity of a neurone with respect to the ventilatory cycle gives a first indication of its possible functions.

(1) *Neurone activity during adduction*

Fig. 2 shows a neurone firing during the complete adduction phase. On records 1 and 3, taken respectively before and after record 2, it is spontaneously active. Record 2 was made while the adductor mandibulae was stimulated electrically with short bursts of pulses during the initial part of the adduction. The result was a decrease in firing frequency. In records 4, 5 and 6, where the pulse intervals of the neurone's spikes are plotted, this can be seen more easily. There every spike is represented by a dot, the height of which is a measure of the time elapsed since the previous spike. The higher the dot the shorter the interval (or the higher the frequency), and the lower the dot the longer the interval (or the lower the frequency).

During the normal activity of the neurone (traces 4 and 6) its firing frequency gradually decreased in the course of every burst. When the adductor mandibulae was stimulated, however, a discontinuity occurred after the first few spikes and the frequency suddenly dropped to a lower level (trace 5).

Another neurone (Fig. 3, records 1 and 4) normally was active during the last part of the adduction. This cell, like that described above, is sensitive to adductor mandibulae stimulation, and although a few other units were also firing occasionally, its spikes can easily be recognized (records 2 and 3). The bursts of normal activity in the

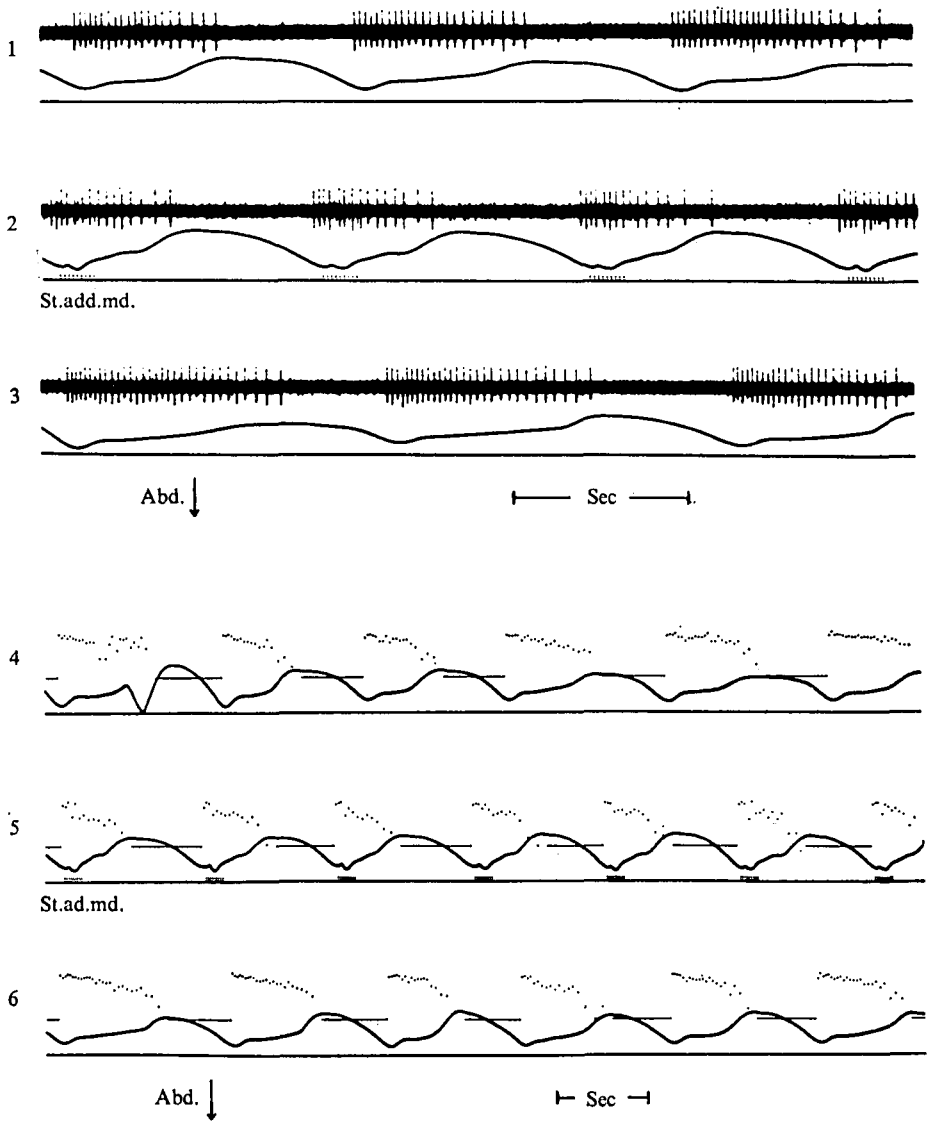

Fig. 2. Activity of a neurone firing during adduction. In every record: first trace = neurone activity, second trace = respiratory movements, third trace = muscle stimulus signal. In records 1, 2 and 3 the neurone activity is shown as a normal spike record, in 4, 5 and 6 as a pulse interval or spike frequency plot. (A higher dot stands for a shorter interval or higher frequency). Records 1, 3, 4 and 6, normal activity. Records 2 and 5, activity during adductor mandibulae stimulation. Reduction of the firing frequency of the neurone, beginning during the stimulus period and persisting throughout the burst (especially clear in record 5).

records are marked with *a*, those following stimulation with *a'*. It is obvious that the neurones responded with a burst of action potentials (coinciding with an inflexion in the movement trace) to stimulation of the adductor mandibulae during any part of the respiratory cycle.

Fig. 4 again shows the behaviour of a neurone that was spontaneously active during the last part of the adduction phase (records 1, 3, 4 and 6). This cell, however, in

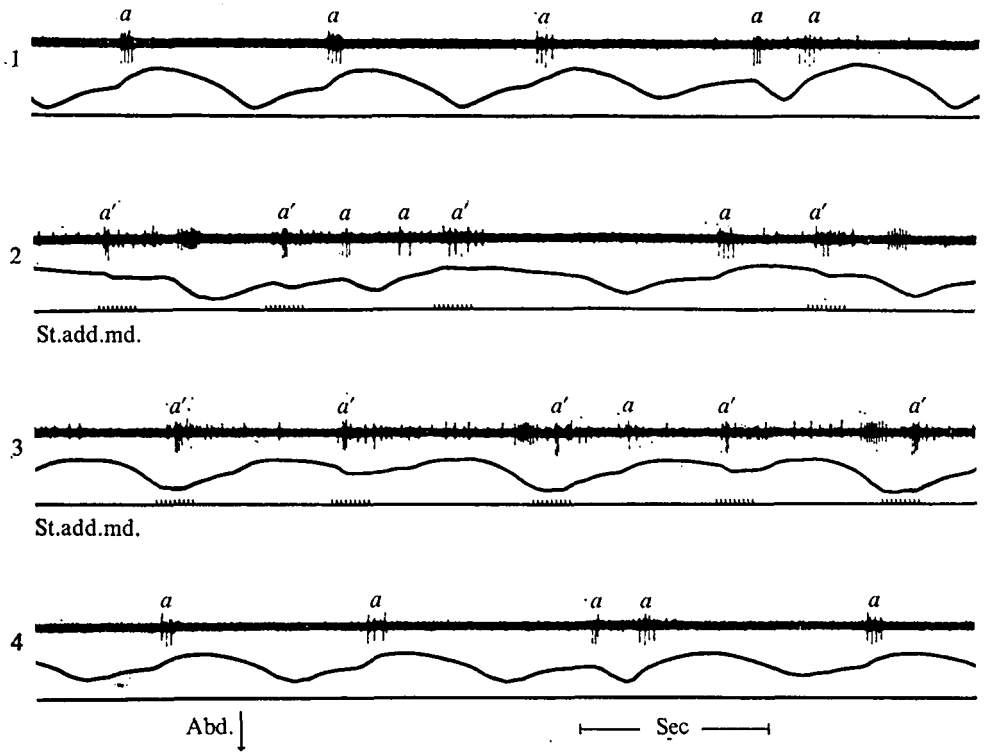

Fig. 3. Activity of a neurone firing during the last part of adduction. In every record: first trace = neurone activity, second trace = respiratory movement, third trace = muscle stimulus signal. Records 1 and 4, normal neurone activity (burst marked with *a*). Records 2 and 3, neurone activity during adductor mandibulae stimulation. (The normal bursts are marked with *a* and those in response to stimulation with *a'*.) Note that in these records several other units with no clear relation to the stimulus are also activated.

contrast to the two described above, responded to stimulation of the adductor mandibulae (record 2) and also to stimulation of the levator hyomandibulae (record 5). In both cases the number of spikes per burst was reduced from 3 to 2. However, to obtain this effect, stimulation of the levator hyomandibulae had to occur during the last part of adduction, in phase with the neurone's activity, whereas stimulation of the adductor mandibulae had to be in antiphase with the neurone, during the end of the abduction.

(2) *Neurone activity during the transition between adduction and abduction*

The neurone of Fig. 5, like that shown in Fig. 4, was sensitive to separate stimulation of more than one muscle, e.g. the adductor mandibulae and the dilator operculi. It is uncertain whether it should be classified as an 'adduction cell' or as a 'transition cell'. In the discussion it will be shown, however, that the distinction for the present purpose is not very important for this particular cell, which was spontaneously active during the later part of the adduction but became transitional during adductor mandibulae stimulation (Fig. 5, records 2 and 3). A stimulus of moderate intensity delivered to this muscle considerably lengthened the burst of activity of the neurone, which then covered the complete abduction phase as well, and ended long after the

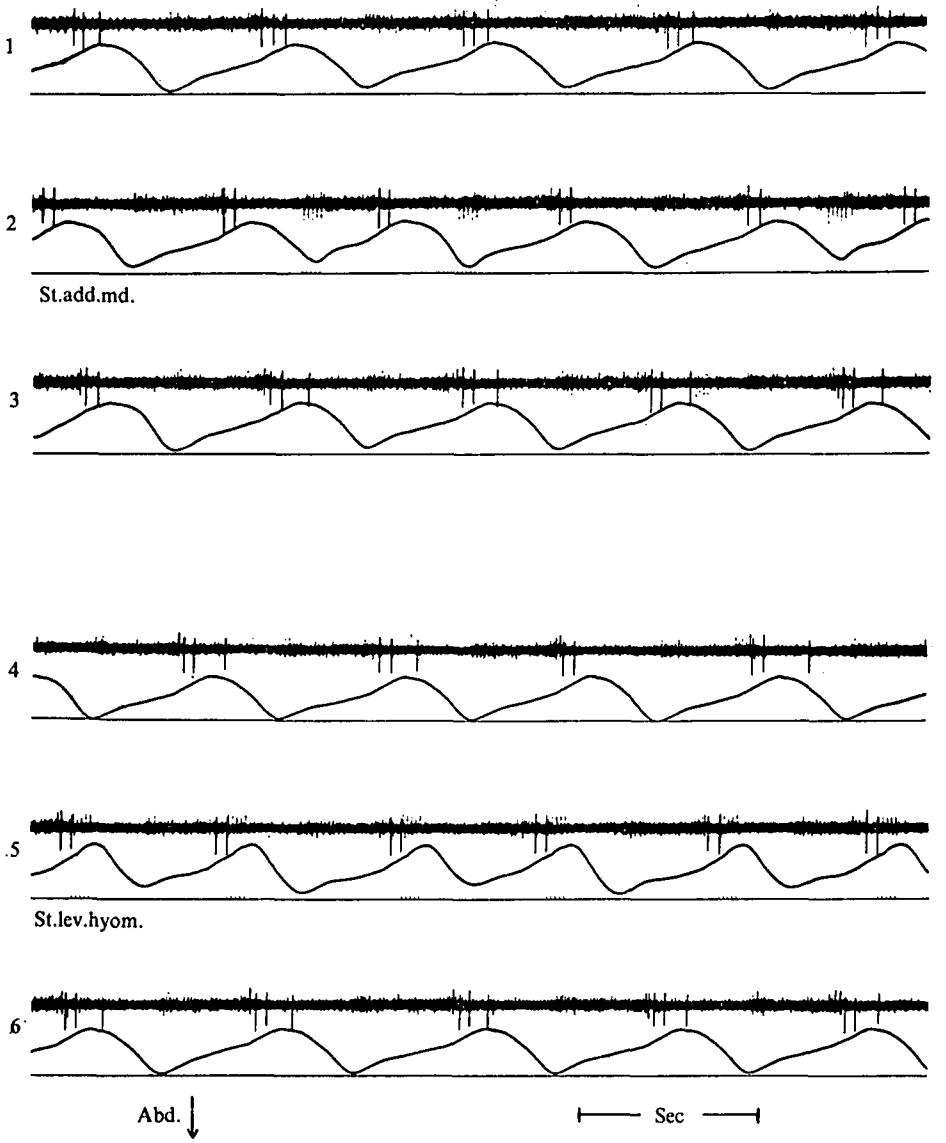

Fig. 4. Activity of a neurone firing during the last part of adduction. In every record: first trace = neurone activity, second trace = respiratory movements, third trace = muscle stimulus signal. Records 1, 3, 4 and 6, normal activity. Record 2, activity during stimulation of the adductor mandibulae in antiphase with the neurone. The number of spikes per burst is reduced. Record 5, activity during stimulation of the levator hyomandibulae in phase with the neurone. The number of spikes per burst is reduced. (In records 2 and 5 a stimulus artifact could not be avoided.)

termination of the stimulus (record 2). When a stimulus of low intensity was given, only one additional spike was fired after a long delay (record 3). Its timing was roughly comparable to that of the last spike of the burst resulting from strong stimulation.

Intermediate intensity stimulation of the dilator operculi, triggered by the first spike of the neurone's activity, inhibited all further action potentials (records 5 and 5*a*, two consecutive rows). Furthermore the effect in this case was cumulative,

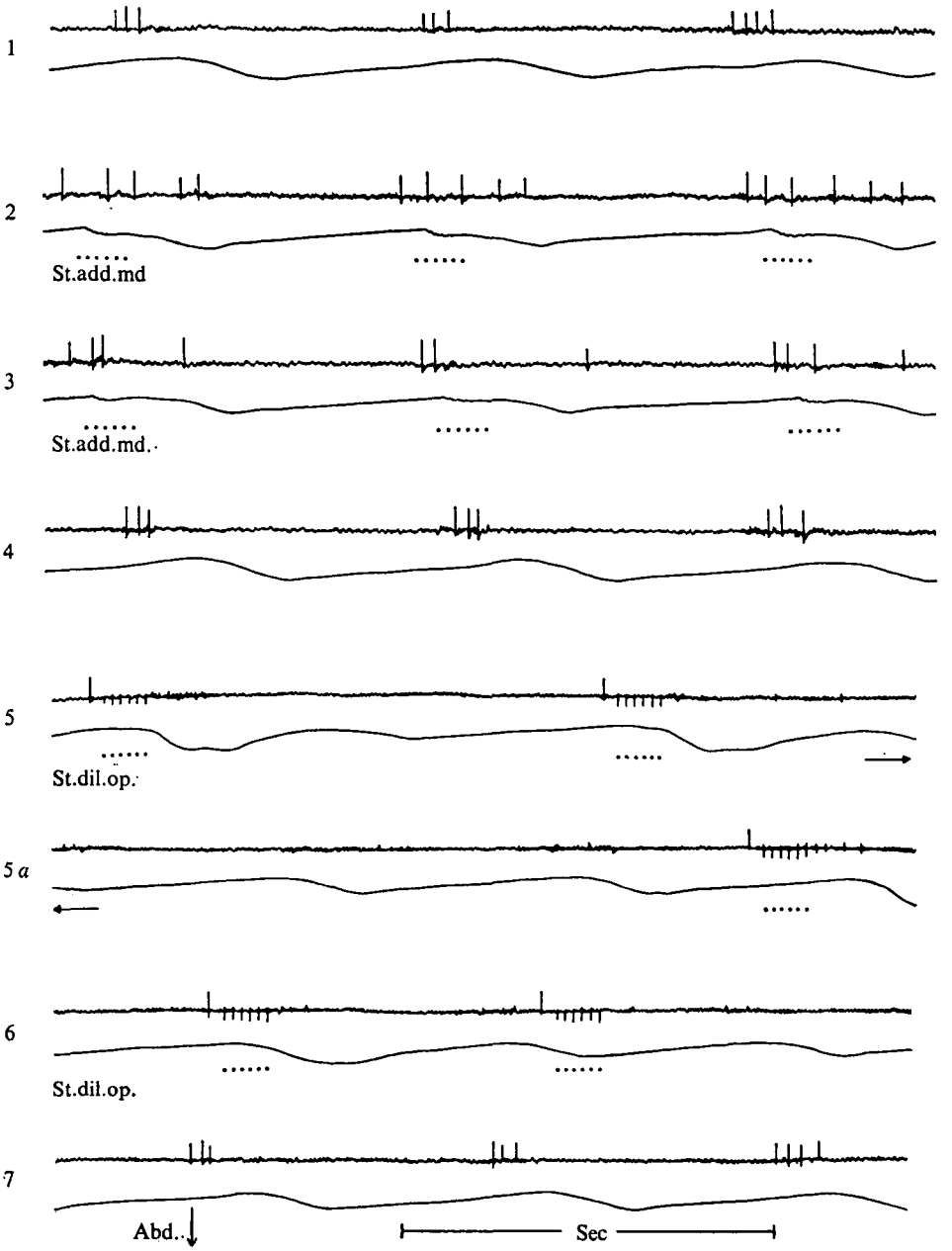

Fig. 5. Activity of a neurone firing during the last part of adduction. In every record: first trace = neurone activity, second trace = respiratory movements, third trace (dots) = muscle stimulus signal. Records 1, 4 and 7, normal activity. Record 2, activity during stimulation of the adductor mandibulae. The number of spikes per burst and the burst length are increased, and the neurone is now firing during the abduction as well. Record 3, activity during weaker stimulation of the adductor mandibulae. One additional spike per burst is fired after a long latency. Record 5 (continuous with 5*a*). Activity during stimulation of the dilator operculi. The neurone is inhibited by the stimulus. The inhibition can last for several cycles in which no stimulus is present. Record 6, activity during weak stimulation of the dilator operculi. Inhibition of the neurone which generally only acts during one respiratory cycle. (In records 5 and 6 a stimulus artifact could not be avoided.)

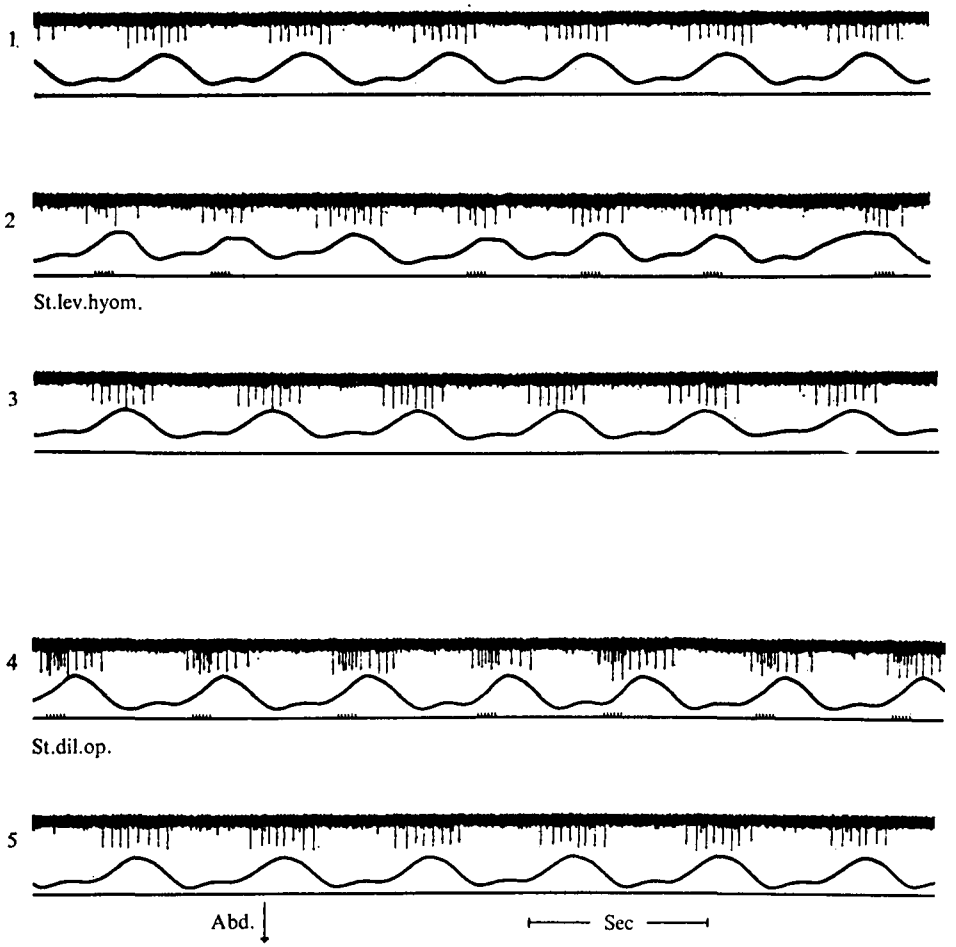

Fig. 6. Activity of a neurone firing during the transition from adduction to abduction. In every record: first trace = neurone activity, second trace = respiratory movement, third trace = muscle stimulus signal. Records 1, 3 and 5, normal activity. Record 2, activity during stimulation of the levator hyomandibulae. The number of spikes per burst is reduced except during the third respiration where no stimulus was given. Record 4, activity during stimulation of the dilator operculi. Neurone activity unchanged. (A stimulus artifact could not be avoided.)

because after a while several respiratory cycles without any activity of the cell (and consequently without stimulus) passed before the cell fired once more. During weaker stimulation this cumulative effect was less pronounced (record 6). Record 7 again shows the spontaneous activity at the end of the experiment.

The neurone of Fig. 6 was clearly transitional in character. Its activity started during the adduction and ended during the abduction phase (records 1 and 3). Stimulation of the levator hyomandibulae (record 2) resulted in shortening of the burst of activity and reduction of the number of spikes. This effect was not the result of a general expansion movement because stimulation of the dilator operculi (record 4) was without any effect.

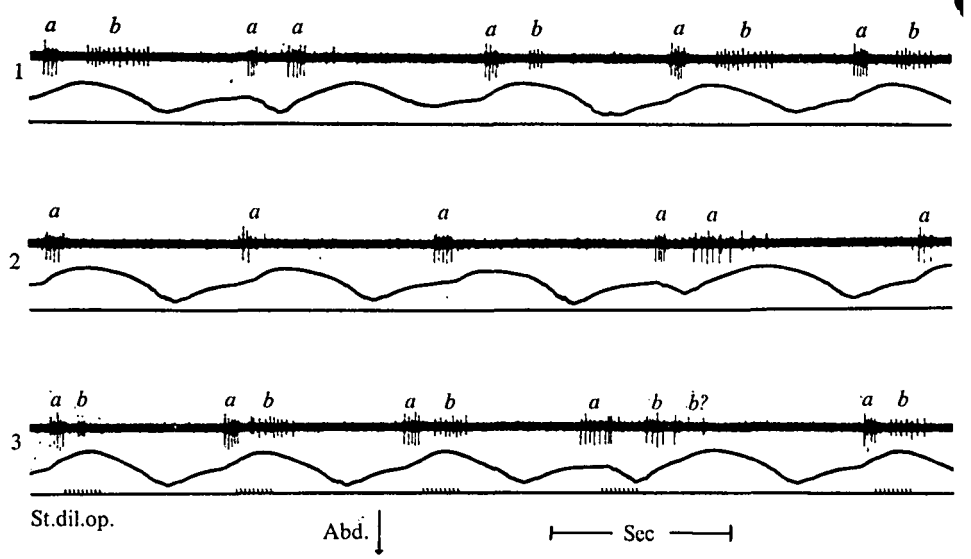

Fig. 7. Activity of a neurone firing during abduction (burst marked with *b*). In every record: first trace = neurone activity, second trace = respiratory movement, third trace = muscle stimulus signal. Records 1 and 2, normal activity. In 1 the cell is spontaneously active, in 2 it is not. Record 3, activity during dilator operculi stimulation. Except during the cough, when the cell is never active (see also trace 1), stimulation of the dilator operculi elicits a burst of activity.

(3) *Neurone activity during abduction*

Fig. 7 shows the activity of two cells recorded together. The action potentials of one cell (marked *a* in the record) coincided with the adduction phase of respiration and do not concern us here. The burst of the other cell, if present, occurred during the abduction (*b* in the record). The neurone sometimes was and sometimes was not spontaneously active (Fig. 7, respectively trace 1 and trace 2). Stimulation of the dilator operculi around the point of maximal adduction of the respiratory system activated the neurone (record 3) except in the middle of a cough as can be seen in the 4th respiration of record 3.

DISCUSSION

Research on the muscle coordination of fish respiration (Ballintijn & Hughes, 1965; Ballintijn, 1969*c*; Hughes & Ballintijn, 1965; Osse, 1969) showed that the elements of the respiratory system are coupled mechanically so that movements of a given part spread over the system. In fact this is one of the basic properties giving rise to the high mechanical efficiency of the respiratory pump in fish (Ballintijn, 1972). For the present investigation in which the proprioceptive reflex effects of contraction of individual muscles are studied, this high degree of mechanical coupling is very inconvenient. It appeared possible, however, to minimize spreading of the influence sufficiently by keeping the stimulus, and thus the twitch of the muscle, small and short.

The movement record taken from the hyomandibula, the best place to obtain an overall impression of the pumping activity because it reflects expansion and contraction of the buccal as well as the opercular cavities, serves only as a general indication

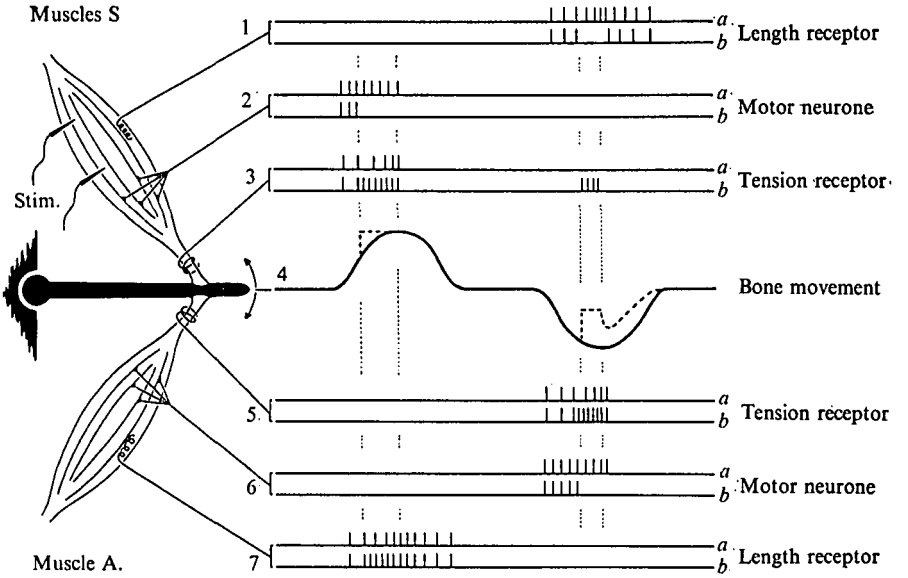

Fig. 8. Schematic representation of the normal firing pattern of the elements of a proprioceptive control loop and their reaction to a short muscle contraction. Muscle S is the muscle that can be stimulated, muscle A is the antagonist of the stimulated muscle. Middle trace (4): movement record. The movement during stimulation is shown by a broken line. Upper three traces: activity of a motor neurone (2), tension receptor neurone (3) and length receptor neurone (1) of muscle S, at *a* during normal activity, at *b* during stimulation of muscle S. Lower three traces: activity of a motor neurone (6), tension receptor neurone (5), and length receptor neurone (7) of muscle A, at *a* during normal activity, at *b* during stimulation of muscle S.

of ventilation. It certainly can not be regarded as a representation of the changes in movement of separate muscles under the influence of stimulation.

The choice of the muscles

The most important respiratory muscles in the carp are:

(1) The levator hyomandibulae and its antagonist the adductor arcus palatini, respectively expanding and contracting the buccal as well as the opercular cavities.

(2) The dilator operculi and its antagonist the adductor operculi, respectively expanding and contracting the posterior part of the opercular cavity and abducting and adducting the operculum.

(3) The adductor mandibulae (part A_3) and its antagonist the levator operculi, respectively adducting and abducting the lower jaw.

It is to these three main groups of respiratory muscles that the present investigation was limited, and the experimental conditions were such that only these muscles took part in respiration.

As will be explained below it is only necessary to stimulate one of an antagonistic pair of muscles to obtain data on the elements of the proprioceptive control system of both, when two experiments are carried out in succession. In one the muscle is stimulated in phase with its normal activity, and in the other in antiphase (i.e. in phase with its antagonist). In the present case, the levator hyomandibulae, the dilator operculi and the adductor mandibulae were selected because they are large, super-

ficial and easy to reach and it is not particularly difficult to stimulate them without problems of electrical leaks to other muscles. In every experimental series used for this paper, all these muscles have been stimulated both in phase and in antiphase with their spontaneous activity.

The properties of proprioceptive elements

The reaction to a muscle twitch that is caused by electrical stimulation can be predicted for motor neurones, tension receptor neurones (tendon organ neurones) and length receptor neurones (muscle receptor neurones) of both the stimulated muscle and its antagonist. It should be stressed that for the muscle receptors of teleosts, owing to their simple anatomical structure, central driving cannot be expected so that they are taken to be of the passive type. The result is summarized in Fig. 8. The middle trace (4) simulates the movement record of a skeletal element to which an antagonistic pair of muscles is attached. For the muscle to be stimulated (S), the upward excursion of the trace stands for contraction and the downward excursion for extension. The movement as it is during stimulation is drawn in a broken line. For the antagonist (A) of the stimulated muscle the meaning of the trace of course is the reverse: extension is 'up' and contraction 'down'. The upper three pairs of traces (1, 2 and 3) show the activity of a motor neurone, a tension receptor neurone and a length receptor neurone of the muscle that can be stimulated (S). Of every pair, the upper one (*a*) represents the normal activity and the lower one (*b*) the activity during muscle stimulation, as given by the broken line in the movement trace (4). The lower three pairs of traces (5, 6 and 7) are from a motor neurone, a tension receptor neurone and a length receptor neurone of the antagonist (A) of the stimulated muscle. Here again the upper trace of a pair is normal and the lower one during stimulation.

During the upward excursion of the movement trace three types of cell are active:

(1) The motor neurones of muscle S, which causes the movement of the skeletal element to which it is connected. They fire during the rising phase of the movement trace and not during the falling phase, the relaxation (trace 2*a*).

(2) The tension receptor neurones of muscle S. They also fire during the rising phase because then the tendon is under tension (trace 3*a*).

(3) The length receptor neurones of muscle A. They fire during both the rising and the falling phase of the movement trace because during all that time the muscle is stretched (trace 7*a*).

Stimulation of muscle S, as illustrated by the broken line in trace 4, speeds up the contraction of this muscle. Because this results in greater tension in its tendon, the tension receptor neurones increase their firing rate (trace 3*b*). This in turn results in autogenic inhibition of the motor neurones of muscle S (trace 2*b*), which as a consequence decrease their firing rate or stop firing. Finally stimulation of muscle S accelerates the extension of muscle A. The firing frequency of its length receptor neurones will therefore rise (trace 7*b*).

During the downward excursion of the movement trace again three types of cell are active:

(1) The motor neurones of muscle A, which now causes the movement. They fire during the falling phase of the movement trace and not during the rising phase, the relaxation (trace 6*a*).

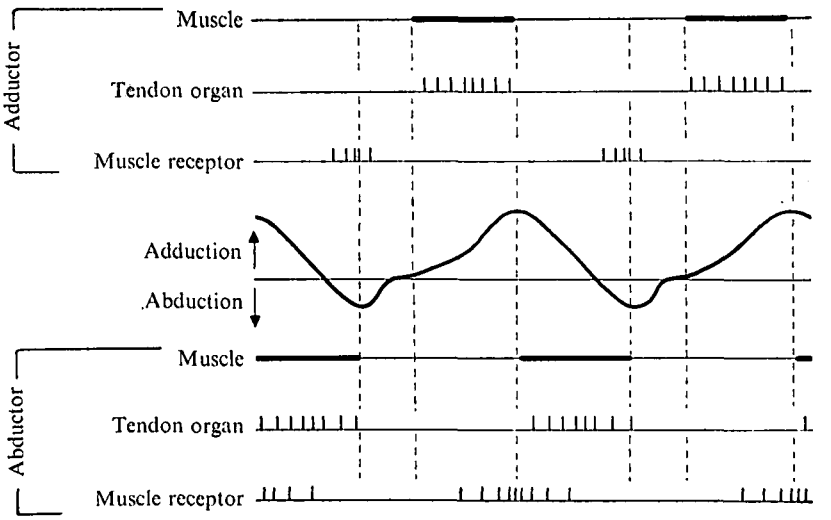

Fig. 9. Hyomandibular movements and the period of activity of adductor and abductor muscles together with the firing pattern of their tension receptor neurones and length receptor neurones, during normal respiration of a carp.

(2) The tension receptor neurones of muscle A. They also fire during the falling phase because then the tendon is under tension (trace 5*a*).

(3) The length receptor neurones of muscle S. They fire during both the rising and the falling phase of the movement trace because during all that time the muscle is stretched (trace 1*a*).

Stimulation of muscle S as indicated by the broken line of trace 4, counteracts the action of muscle A. This in the first place results in an increased tension in the tendon of muscle A and thus in an increase in the firing frequency of its tension receptor neurones (trace 5*b*). This increased activity of the tension receptor neurone also, as a secondary reaction, brings about a decrease in activity of the motor neurones of muscle A through autogenic inhibition (trace 6*b*). Because the stimulus duration is kept short, matters will not be complicated by the drop of tension this behaviour initiates. As the forces of muscle A and muscle S which now both contract, are acting in opposition, tension will also develop in the tendon organs of muscle S, and activate their neurones (trace 3*b*). Further, stimulation of muscle S will decrease the extension forced upon it by the action of muscle A. Consequently the length receptor neurones of muscle S will diminish their activity or stop firing (trace 1*b*).

Finally Fig. 9 shows the time relations of the activity of all the elements described above during the normal respiratory movements of a carp. It should be noted, however, that some adductor muscles normally do not fire during the whole adduction phase but only during the last part of it. This is not shown in the generalized diagram of Fig. 9.

The experimental data

All the neurones which alter their firing pattern in response to the twitches caused by muscle stimulation are, in one way or another, under the influence of proprioceptive information. Analysis of the experimental data presented in the Results section will lead to a better understanding of the different ways in which these influences act

and of the nervous elements that are involved. It is of special interest to investigate whether neurones exist with the properties of the elements of a peripheral proprioceptive control loop as indicated above.

(a) Neurones processing proprioceptive information at a higher level of integration

In the first place, the records show a group of nerve cells which react to separate stimulation of two or more muscles which are not members of an antagonistic pair. This situation is shown in Figs. 4 and 5 where two different neurones react to stimulation of the adductor mandibulae and of the levator hyomandibulae and to stimulation of the adductor mandibulae and of the dilator operculi respectively. As these muscles are not antagonists and movements caused by adductor mandibulae contraction are in no way coupled to movements resulting from levator hyomandibulae or dilator operculi activity (as explained in Ballintijn, 1969*a*, *c*), both neurones under observation obtain their proprioceptive information from at least two different sources. It may be concluded therefore that they are not part of a peripheral proprioceptive control loop but process proprioceptive information at a higher level of integration.

This view is corroborated by other evidence. Delayed reactions of the neurone to the stimulus and an after-effect of stimulation lasting for several respiratory cycles can occur. Neither would be expected in a direct proprioceptive control loop.

The first case is illustrated in Fig. 4, trace 2, where a short contraction of the adductor mandibulae during the end of the abduction phase resulted in a reduction of the number of spikes in the activity burst of a neurone which fired during the late adduction. A comparable effect can be seen in Fig. 5 during adductor mandibulae stimulation. A strong stimulus (trace 2) in this case resulted in prolongation of the burst of activity of the neurone under observation up to a long time after the contraction had finished. Lower intensity stimulation (trace 3) gave rise to one extra spike which, however, occurred well after the end of the stimulus.

In-phase stimulation of the dilator operculi (Fig. 5, trace 5) produced a strong after-effect on the activity of this same neurone. Dilator operculi contractions inhibited the neurone and in the course of a few respiratory cycles an after-effect built up which stopped it completely for a number of cycles.

Together, the data presented above allow the conclusion that these neurones are not part of a direct proprioceptive control loop but process proprioceptive information at a higher level of integration. The fact that long-term effects occur suggests that these cells play a role in determining future respiration on the basis of proprioceptive signals integrated over a period of time. The limited data available at present, do not justify further speculations on the characteristics and function of this group.

(b) Neurones reacting as elements of a peripheral proprioceptive control loop

In the remaining experiments the neurones under observation did not react to twitches of muscles of more than one antagonistic pair. (As mentioned before, for every neurone investigated, muscles of all three important antagonistic pairs have been stimulated, both in phase and in antiphase.) Thus they satisfy the first criterion for elements of the peripheral proprioceptive control loop. On the grounds of their firing characteristics and responses to muscle stimulation, it can be shown that most

these neurones exhibit the typical properties expected of length receptor neurones, tension receptor neurones or motor neurones.

Fig. 6 shows records of a neurone that behaved as a length receptor of the levator hyodibulamane. Its spontaneous activity (records 1, 3 and 5) occurred during the transition of adduction to abduction, and, as explained before (Figs. 8 and 9), length receptors fire either during the transition from adduction to abduction or during the transition from abduction to adduction when their muscles are extended. Moreover, stimulation of the levator hyomandibulae during its extension resulted in decreased activity of the neurone (Fig. 6, record 2). As explained above (Fig. 8), this is a typical reaction for a length receptor neurone. That this reaction is not the result of general expansion movement can be concluded from the fact that stimulation of the dilator operculi, which also causes considerable expansion of the respiratory cavity, did not influence the firing pattern of the neurone at all (Fig. 6, record 4). The large difference in shape of the movement trace of records 2 and 4 is explained by the fact that the transducer was recording hyomandibular movements. These of course are very sensitive to levator hyomandibulae contractions, while dilator operculi contractions, although giving rise to marked expansion movements, influence the position of the hyomandibula to a much lesser extent. It is therefore justifiable to conclude that the neurone of Fig. 6 behaved as a length receptor neurone of the levator hyomandibulae.

The neurones of Fig. 3 and 7 behaved as tension receptor neurones of the adductor mandibulae and the dilator operculi respectively. The spontaneous activity of the first one (Fig. 3, records 1 and 4) occurred during the last part of the adduction phase, when the activity of the adductor mandibulae, which often starts at a low level, generally shows a marked increase. Thus the timing of the normal burst of the neurone was in agreement with what is expected for either a tension receptor neurone or a motor neurone. However, electrical stimulation of the adductor mandibulae during all phases of the respiratory cycle elicited a series of action potentials from the neurone (Fig. 3, records 2 and 3; a = normal burst, a' is burst in response to stimulation). This reaction rules out the possibility of a motor neurone, since this would be inhibited by stimulation of its own muscle. The reaction is, however, identical to that of a tension receptor neurone, which would fire whenever the tension in the tendon is high, due to either normal muscle contractions or to a twitch following electrical stimulation. (Burst a', resulting from muscle stimulation in the majority of cases, is shorter than the duration of the stimulus, but so is the disturbance of the movement trace. Apparently movements compensating the tension-effect of the stimulus occur.)

The neurone of Fig. 7 appeared to have a higher threshold than that of Fig. 3. If it was spontaneously active it fired during abduction (Fig. 7, record 1, burst b), but generally it was silent during normal respiration (record 2). In those cases where it was active, the movement trace was slightly steeper, indicating that the application of more muscle power was producing a faster movement. The firing pattern thus resembled that of a motor neurone or a tension receptor neurone. Stimulation of the dilator operculi, except during the cough when the neurone never fired, elicited a burst of action potentials (Fig. 7, record 3), so that here also the possibility of a motor neurone is ruled out and the neurone appears to have been a tension receptor neurone of the dilator operculi.

Finally in Fig. 2 an example of a motor neurone is given. The normal activity of the

cell occurred during the adduction phase when both motor neurones and tension receptor neurones were firing (records 1, 3, 4 and 6). In this experiment, electrical stimulation of the adductor mandibulae resulted in a decrease in firing frequency of the neurone, which shows very clearly in the pulse interval plots of Fig. 2 (record 5). Thus this cell behaved as a motor neurone and not as a tension receptor neurone. After termination of the stimulus the firing frequency of the neurone did not return to its original level. It continued from the new level because part of the movement, being adduction of the lower jaw, had already been performed due to stimulation.

Conclusions

Proprioceptive signals are processed in the respiratory centre in the medulla oblongata of the carp. This processing is of two kinds. At a comparatively high level of integration, signals of several sources converge and after-effects of medium or long duration make possible 'advance programming' of respiration. At a lower level, proprioceptive information is processed in proprioceptive control loops containing nerve cells with the properties of length receptor neurones, tension receptor neurones and motor neurones. With the aid of these networks, breath-by-beath disturbances of the respiratory movements can be quickly compensated.

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